Mestrelab Research

Fraction Analysis 1.0

STARTING GUIDE

Document Number
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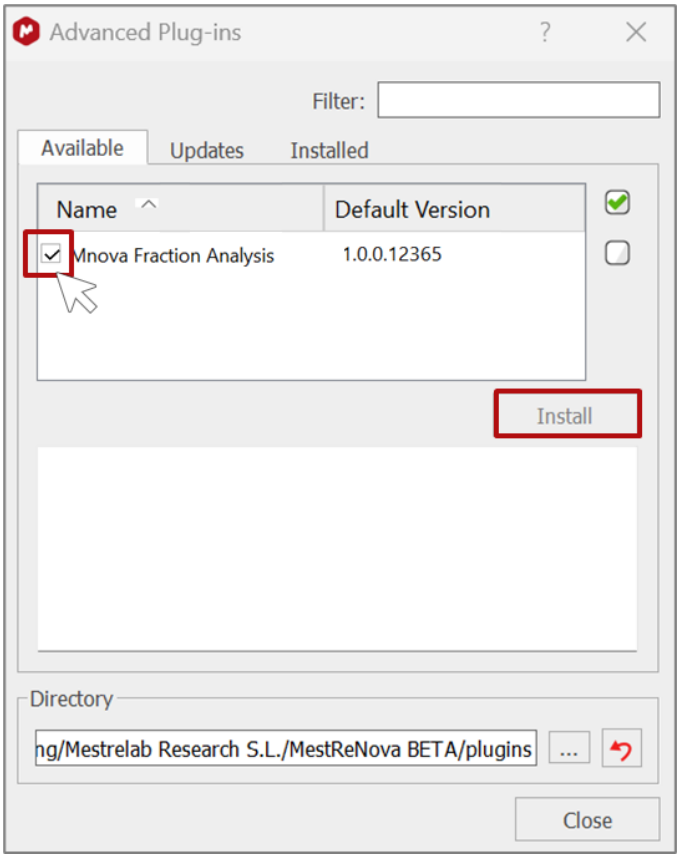
Fraction Analysis is a plugin for Mgears designed to analyze preparative LCMS data to decide as to whether fractions are worth keeping or should be disposed of. It achieves this by considering the mass spectra of the collected fractions as well as the characteristics of the UV peaks associated with them. Using user-defined criteria, it sorts fractions into one of three categories: QC (send to quality control), dispose (unlikely to contain sufficient, or sufficiently pure, product), or review (data is ambiguous and should be reviewed by an analyst). An expert can quickly review the analysis for a batch in the Mgears Viewer and reassign categories in a straightforward manner.

This document is designed to help you get started with Fraction Analysis to streamline decision making and enhance the efficiency of your purification workflows.

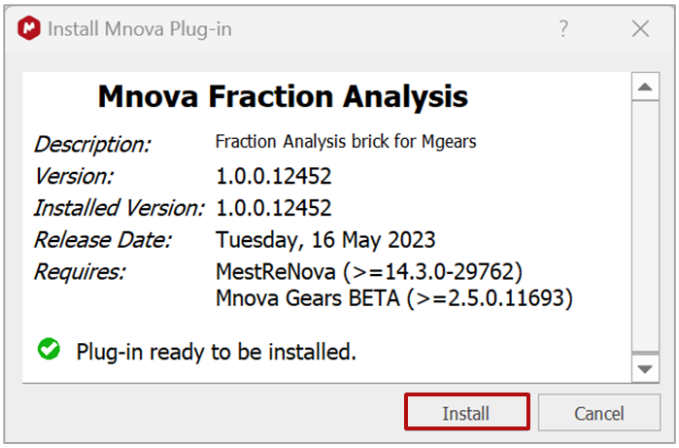
1. Installation

Before installing Fraction Analysis, make sure you already have Mnova MSChrom (minimum version: 15.0) and Mgears (minimum version: 2.5) installed and running with valid licenses.

Go to **Files>Advanced Plug-ins>Available**. Tick the Fraction Analysis plugin, then press **Install**.

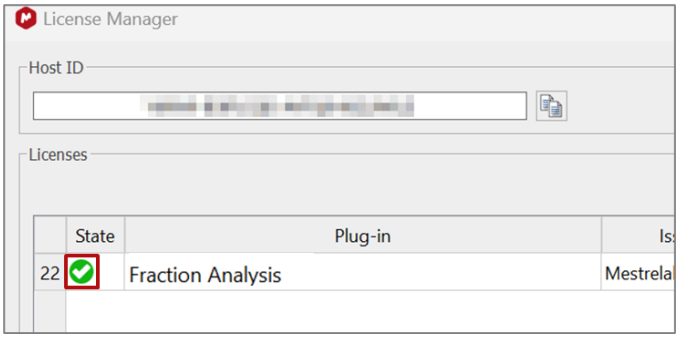


Another option is to drag and drop the Fraction Analysis installer into the Mnova interface. Mnova will report whether all dependencies are resolved, notify you of the version you are about to install, and give you the option to proceed or cancel the installation. If you proceed with the install, you will need to restart Mnova to use the software.



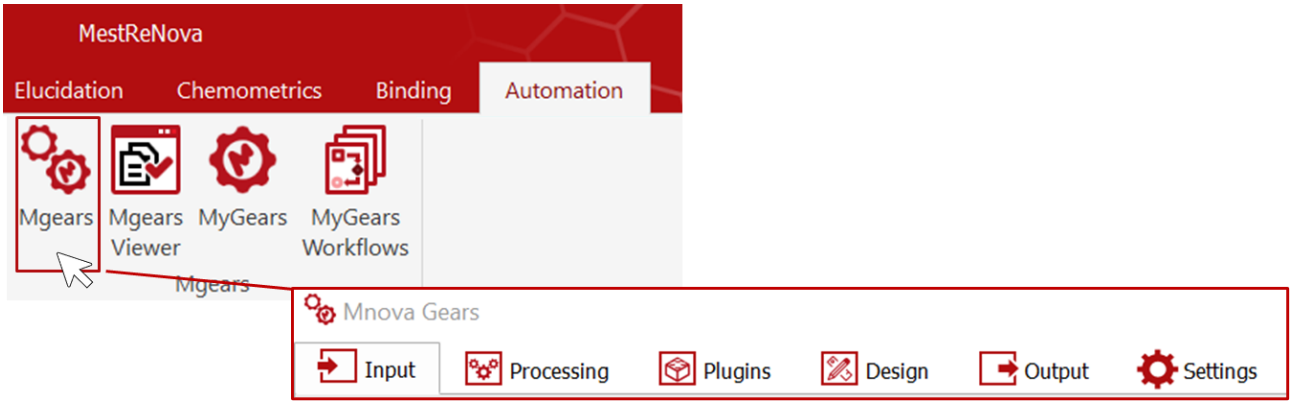
Restart Mnova.

Fraction Analysis must now be installed. You can check your license status by going to **Files>Help>License Manager.** A green check must appear in the plugin's status column.



2. The workflow

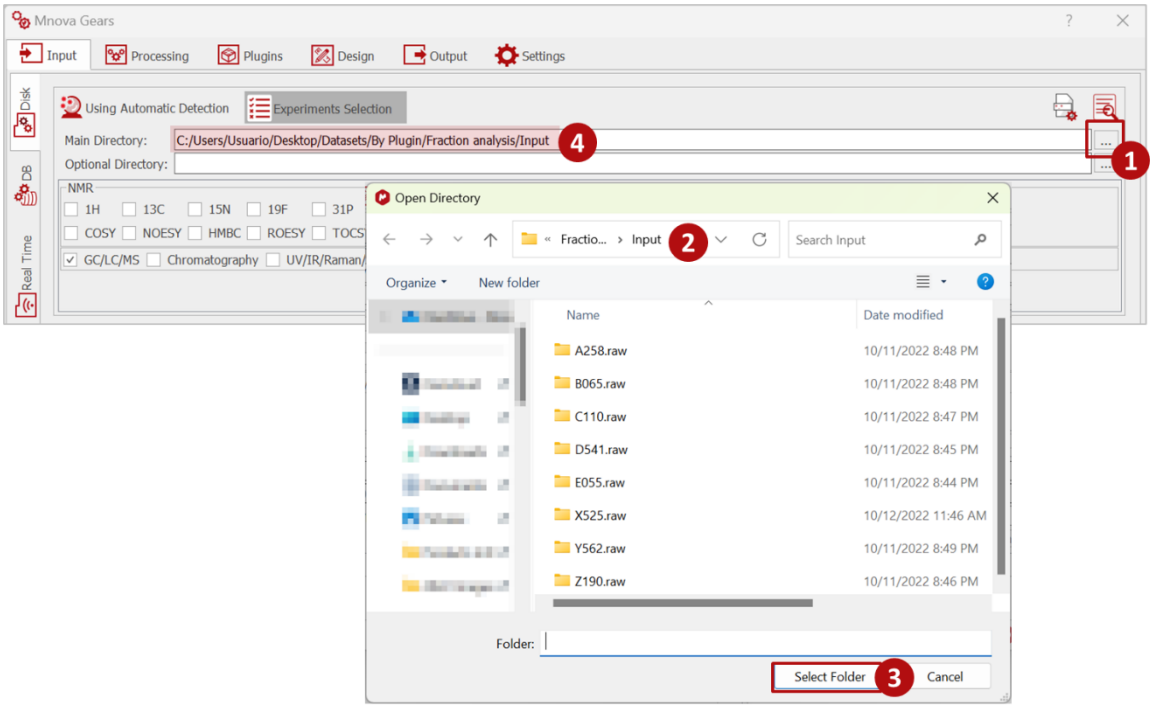
Launch Mgears from the Mnova **Automation** ribbon. The dialog with the usual six tabs will open.



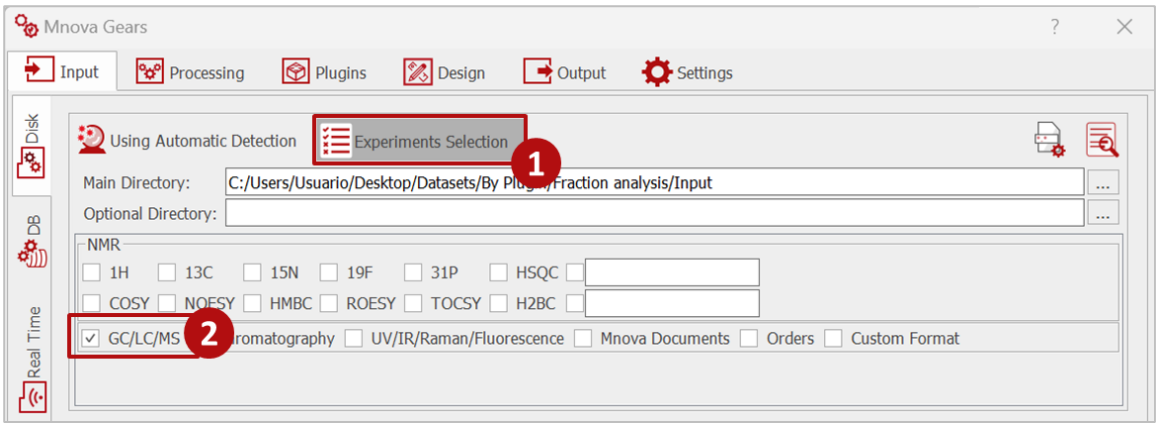
2.1. Input

Fraction Analysis runs from within Mnova Gears and therefore follows the general setup workflow, starting with the input data. LCMS data can come from your local **Disk**, **Database**, or from **Real-Time** acquisition. In this guide, we will work with data from disk directories *(please refer to the Mnova Gears manual for more detail about other input types).*

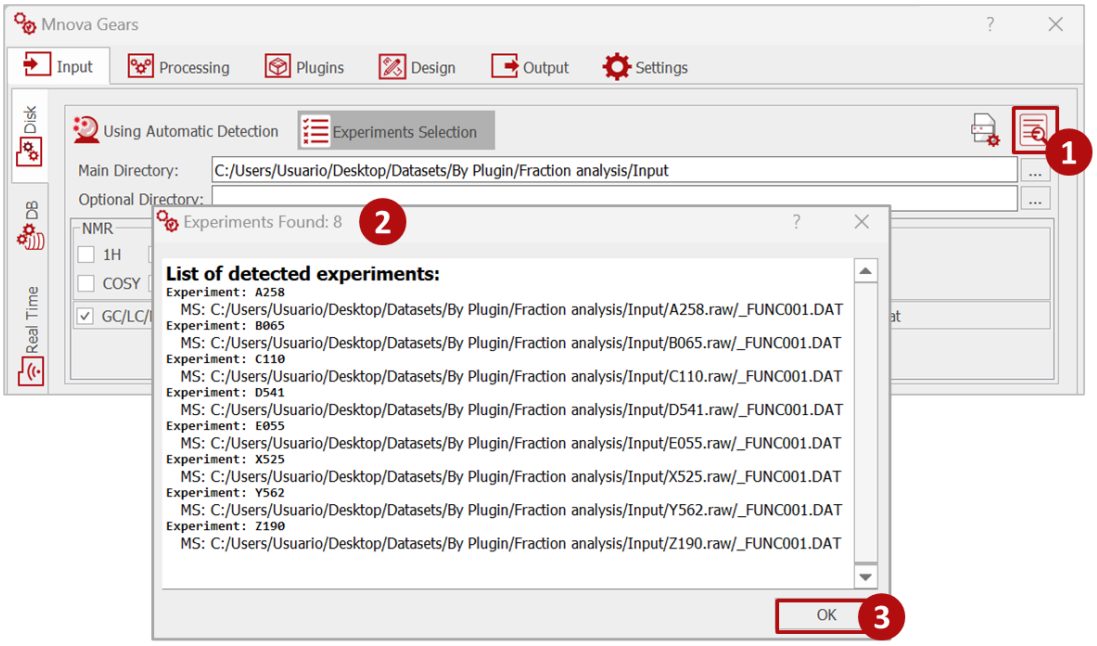
Click on the ... button and select the data files to upload as your **Main Directory**.



The detection of experimental files can be achieved manually by selecting the experiment type(s) and providing **Path Masks** to the relevant data, or automatically via Mgears. When using the **Automatic Detection** mode, **Experiment selection** is recommended if your data folder contains different types of data files to restrict detection to GC/LC/MS and avoid analysis of other undesired files. In this case, you must select the **GC/LC/MS** experiment type, as shown below.

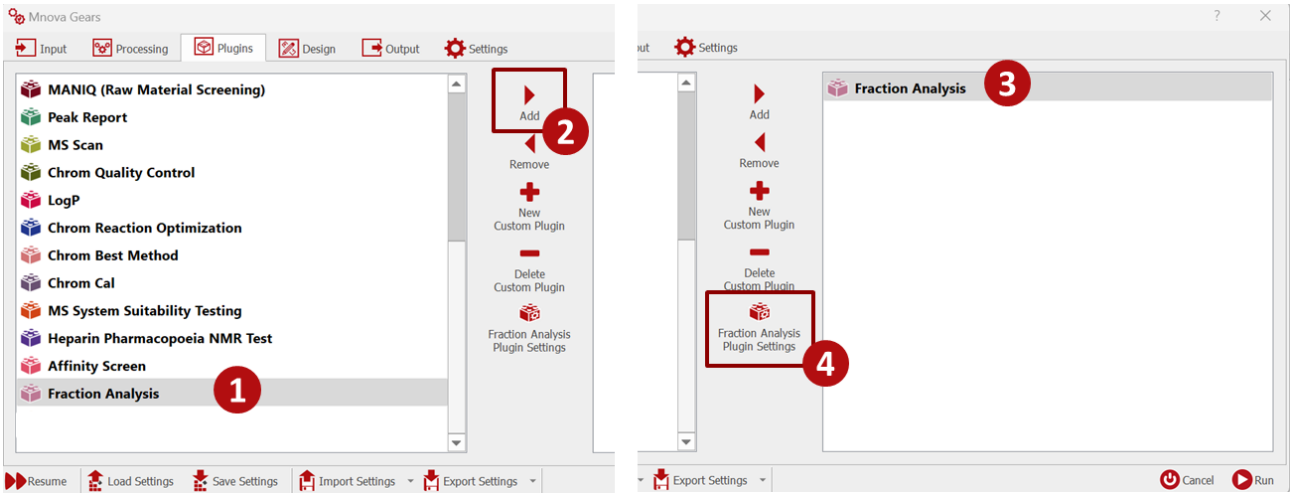


You can now perform an automatic inspection of the selected directory by clicking on  to check whether your input files are found and filtered correctly.



2.2. Plugins

In the **Plugins** section, select and add the Fraction Analysis plugin. Then, click on **Fraction Analysis Plugin Settings** to configure your analysis and reporting method.



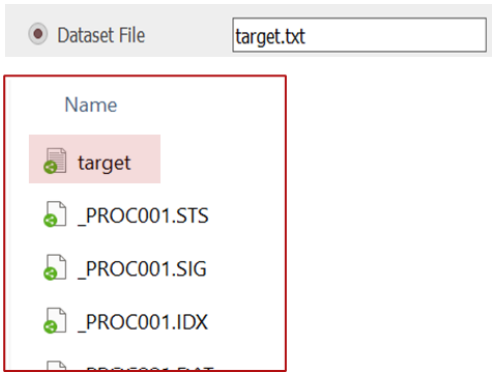
A dialog with three tabs should appear: the **Input**, **Analysis**, and **Quality Controls** tabs.

2.2.1. The Input tab

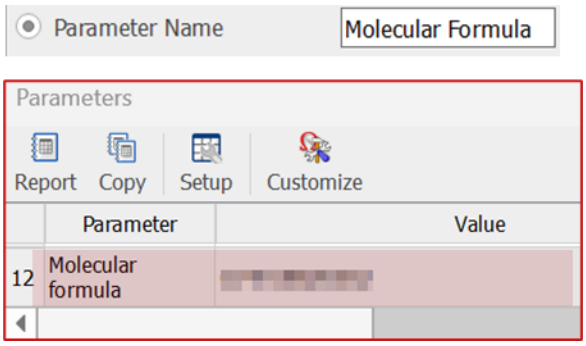
In the **Input** tab, you must choose how the target will be searched for. Target identification can be achieved in various ways:

① **Structure in document:** If you choose this option, Fraction Analysis will use the molecular structure provided within the data document as the target compound.

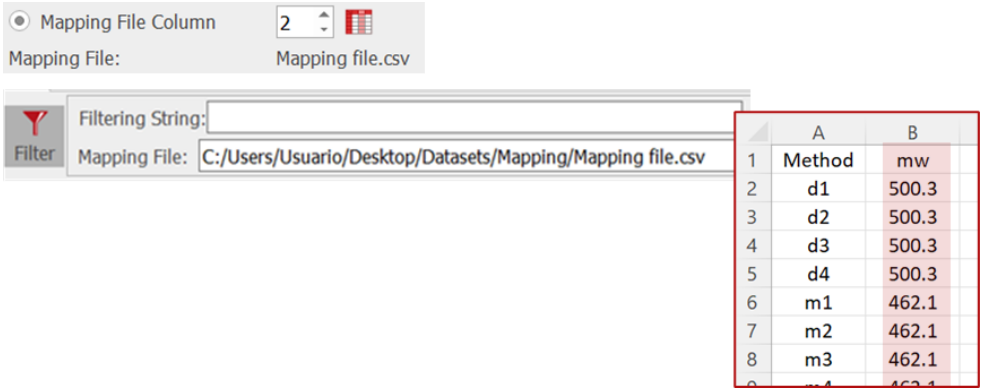
② **Dataset Filename:** When you select this option, the target's molecular formula, molecular weight, or m/z ratio will be extracted from a text file stored within your datasets. You will need to provide the file name to search for in the dataset.



③ **Parameter Name:** With this choice, the target's molecular formula, molecular weight, or m/z ratio will be retrieved from the dataset parameters. You must specify the Mnova parameter name to be used for this purpose. It is important to note that this should be the parameter name as recognized within Mnova, not its "native" name in the original data. Please refer to the note below for instructions on configuring this in Mnova.

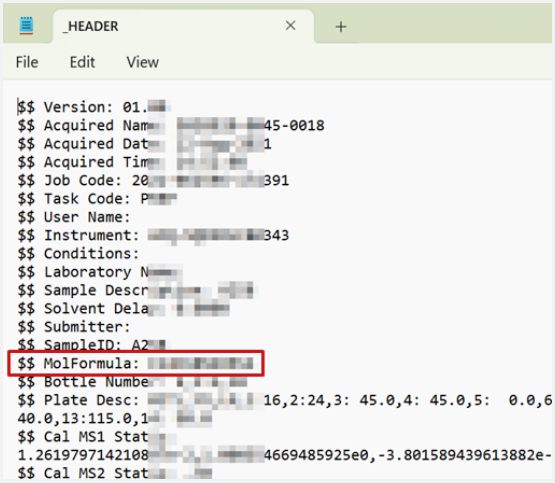


④ **Mapping File Column:** If you opt for this method, the target's molecular formula, molecular weight, or m/z ratio will be retrieved from a CSV file. Be sure to include the CSV file on the Input tab (Mapping file). Here, you will need to indicate which column contains the mass or formula information.



Prerequisites for target information extraction from parameters

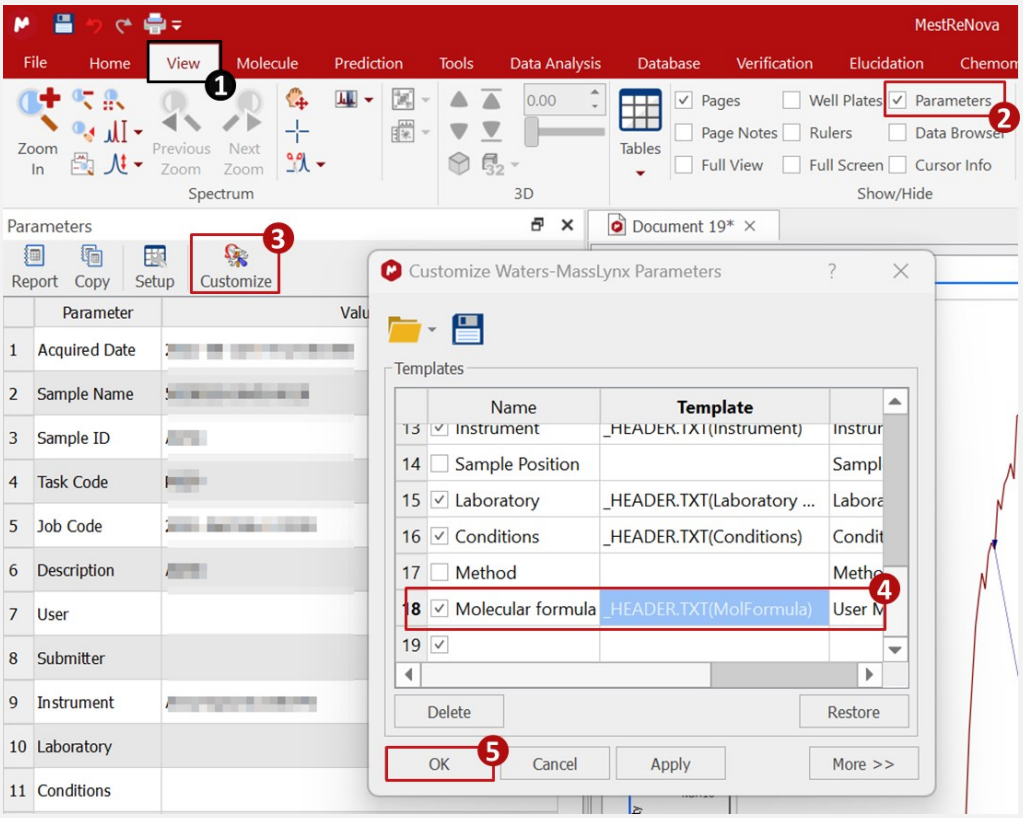
This is a convenient way to supply the workflow with target information, assuming you have it in your prep files. It may well be present as a parameter called 'target mass', or something similar. To make this information available to the brick, you need to set up an Mnova parameter to parse it from the input data.



Open your dataset in Mnova. Go to the **View** tab and check the **Parameters** option. In the **Parameters** window, click on **Customize**. Enter your parameter name and template as shown in the example below.

Please be aware that the specific parameter you need will depend on your setup. You may need to examine your particular dataset to determine the required parameter.

It is also important to note that parameter customization is specific to your data provider. For instance, if you have a combination of Waters and Agilent instruments, you will need to configure parameters for both accordingly.



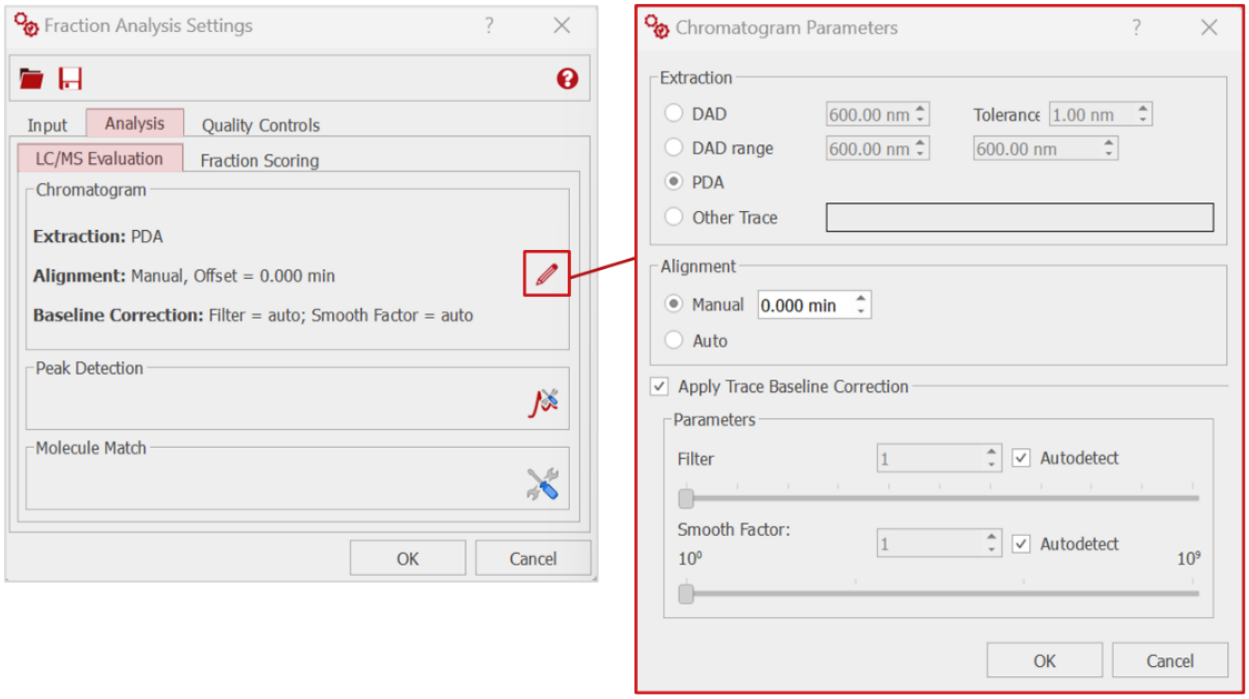
2.2.2. The Analysis tab

2.2.2.1. The LC/MS Evaluation tab

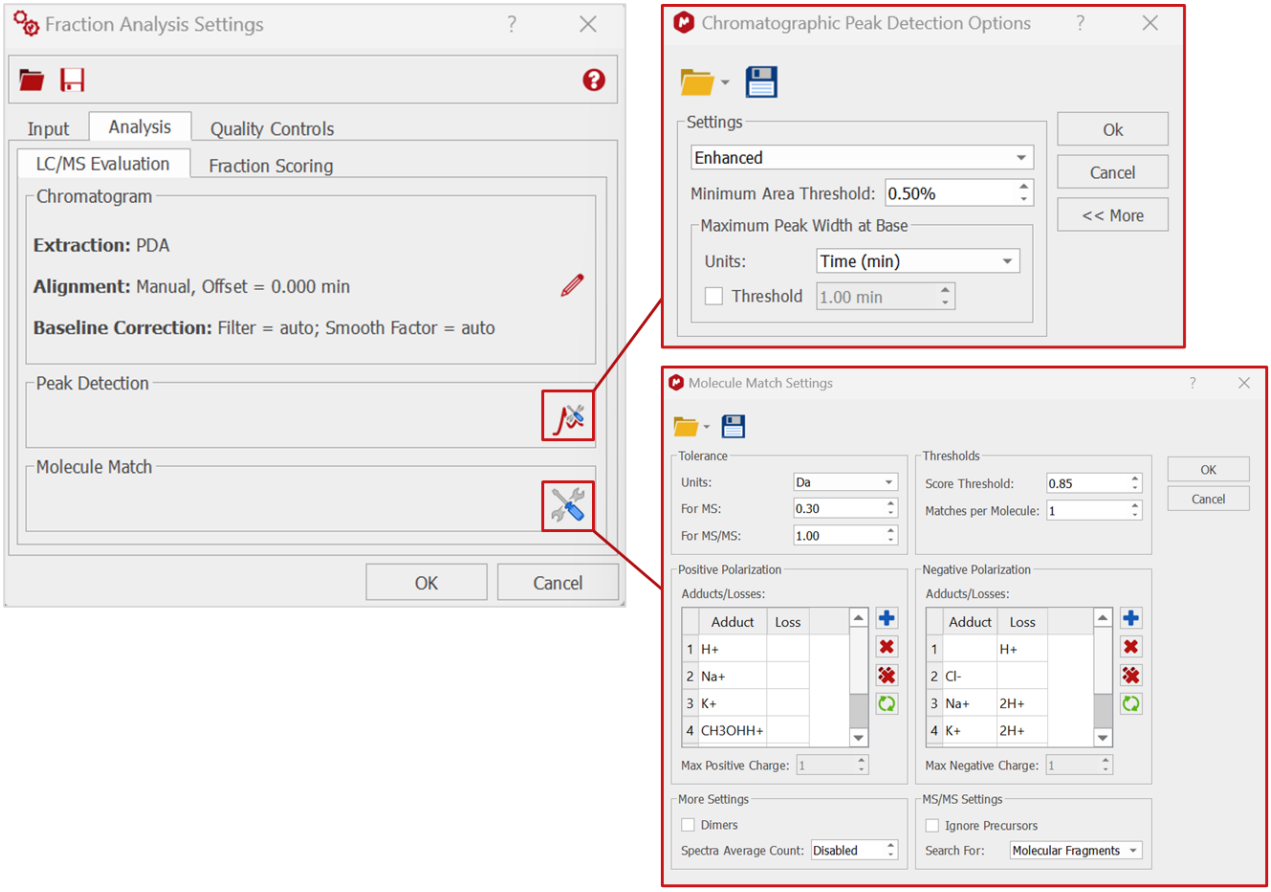
In the **Chromatogram** section you can define which signal will be used for quantification and peak detection. Press the **Add** or **Edit** button to configure the chromatogram extraction preferences.

A dialog will open. Select the appropriate analytical trace type (DAD, DAD range, PDA, or other trace) for chromatogram extraction. Then, you should choose whether to align the chromatograms automatically or manually; we recommend the use of manual alignment. In such a case, the value introduced will be added to the chromatogram in order to align it with the TIC. *(Please refer to this article to see how you can calculate the time-shift required to align chromatograms manually with Mnova.)*

Press **OK** to save your choices.



In this tab, you also have the flexibility to configure the specific **Peak Detection** and **Molecule Match** settings for your analysis without having to modify the generic Peak Detection and Molmatch settings in Mnova. *(Please refer to the Mnova manual for a detailed description of these options).*



The **Molecule Match** settings are specifically utilized for the identification of adducts, which will play a crucial role in evaluating **Peak MS Purity** as detailed in section 2.2.2.2. This means that the better such adducts are defined, the better the purity evaluation.

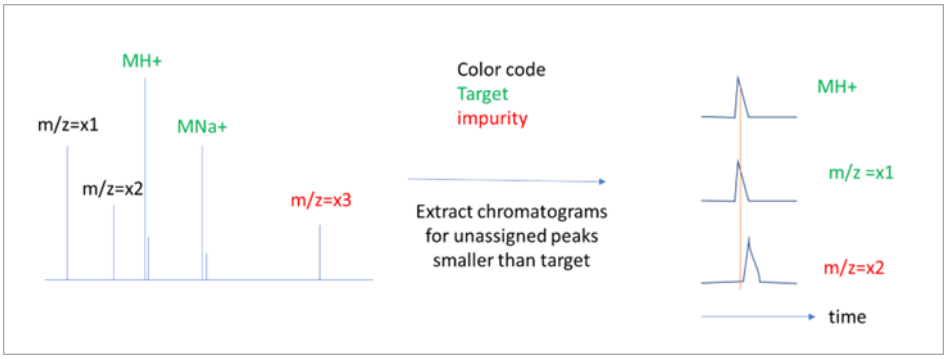
Other **Molecule Match** settings, such as **Thresholds** or **Tolerance**, currently have no impact on the results.

2.2.2.2. The Fraction Scoring tab

Fraction scoring is based on two measurable parameters: the purity and quantity of the analyte in the collected fraction. In this tab, you will need to set the lower and higher thresholds for the evaluation of these two parameters.

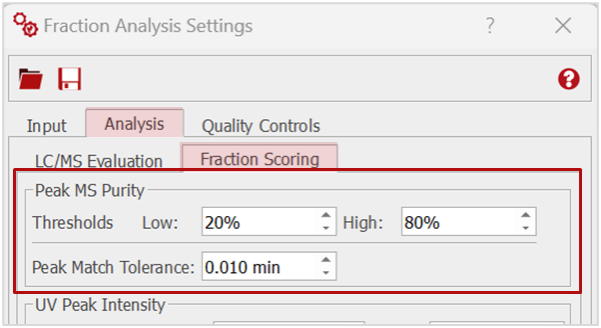
Peak MS purity

- The purity of the analyte is determined by analyzing the masses that are eluted in the fraction. This is achieved by generating an MS spectrum, then matching and assigning the target peaks.
- Adducts that are defined in the Molecule Match table are also considered part of the target.
- Peaks that are larger than the target peak and are not adducts are categorized as impurities.
- Peaks that are smaller than the target peak could also be fragments that need to be considered when calculating purity. To assess this, the tool analyzes the retention time (RT) of the extracted ion chromatogram (EIC) for each of these masses in comparison to the target RT. You can configure the **Peak Match Tolerance** for this evaluation:
    - If the peaks' RTs align with that of the target (within the specified tolerance), they are considered fragments of the target rather than impurities.
    - If the peaks' RTs do not match the target, they are treated as impurities, similar to larger masses.



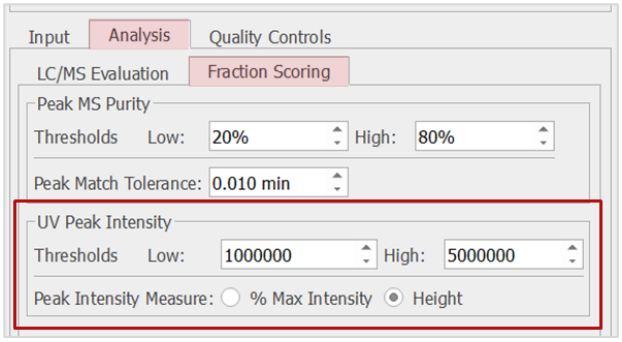
- Purity is then calculated using the formula: $Purity = \frac{target}{target + \sum impurities}$

To evaluate and assign a score for **Peak MS Purity**, it is necessary to set “low” and “high” scoring thresholds as a percentage of the maximum peak MS purity. By setting these thresholds at 20% and 80% as in the image below, a sample would be considered to pass the test if it has a peak purity of ≥ 80%; conversely, it would fail if its purity is ≤ 20%. Fraction Scoring and decision logic are described in more detail in section 5.



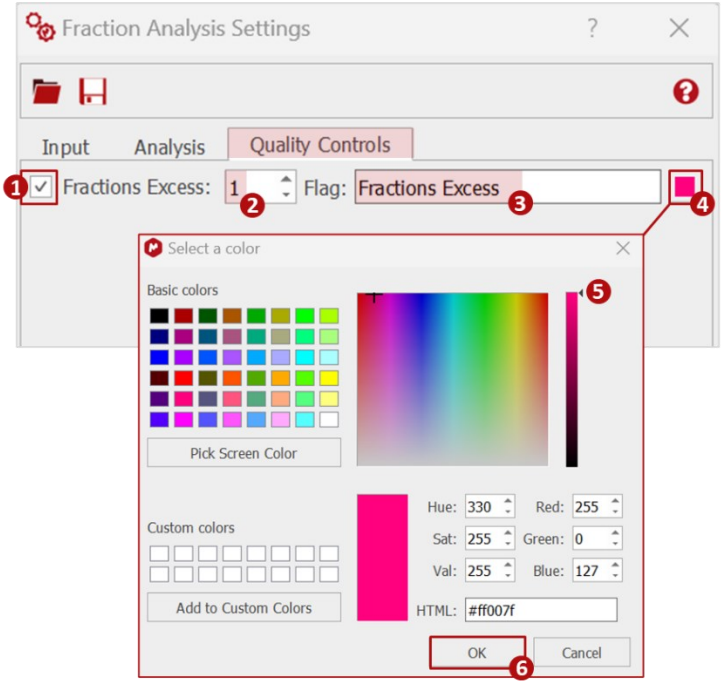
UV Peak Intensity

The quantity of the analyte is determined by the **UV Peak Intensity** for each fraction. The **UV Peak Intensity** "low" and "high" thresholds can be configured as a percentage of the maximal peak height or as an absolute value of the peak height. Fraction Scoring and decision logic are described in more detail in section 5.



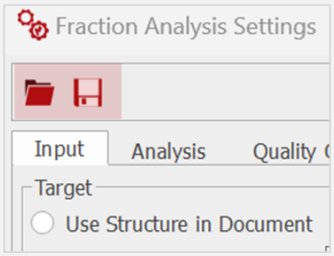
2.2.3. The Quality Controls tab

In the **Quality Controls** tab, you can enable and configure the **Fractions Excess** option to flag up when the number of collected fractions exceeds the desired threshold. This allows you to quickly detect cases where a large number of fractions have been collected.



Finalize your plugin settings setup by pressing **OK** and move to the next step.

Top tip! The analysis settings you have just configured can be saved and reused in future analyses. Press the **Save** button on the top left side of the settings dialog box, then choose a location and press **Save**.

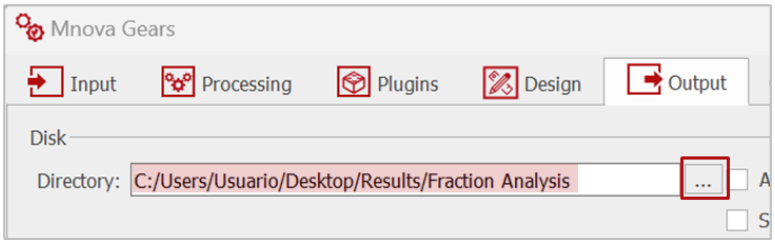


The next time you need to run Fraction Analysis, you only need to press the folder button on the top left side of the settings dialog box, choose your settings file (*.data file) then press **Open**. Your saved settings will be loaded into the settings dialog. All you need to do now is to click OK and move to the next steps.

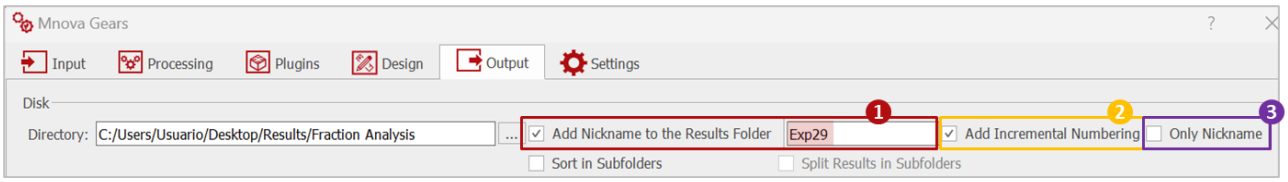
Now, if you are happy with the configuration you can click on **OK**, finalize your plugin settings setup, and move to the next step.

2.3. Output

In the **Output** tab, you will need to choose a directory in which to save your analysis results. Click on ... and select a results folder on your disk.



Optionally, enable the **Add Nickname to the Results Folder** and type the nickname of your choice (1), **Add Incremental Numbering** to your results folder (2), and/or decide whether to use **Only the Nickname** in the folder's name (3).



You can also choose to create an Mnova document, a PDF, or to save your results to a database.

Note. We strongly advise enabling the option to generate **Mnova** document output, particularly if you intend to examine or review the spectra alongside the results in the Mgears Result Viewer. Without this option enabled, when you load the results in the Mgears Result Viewer only the data will be visible, without any spectra.

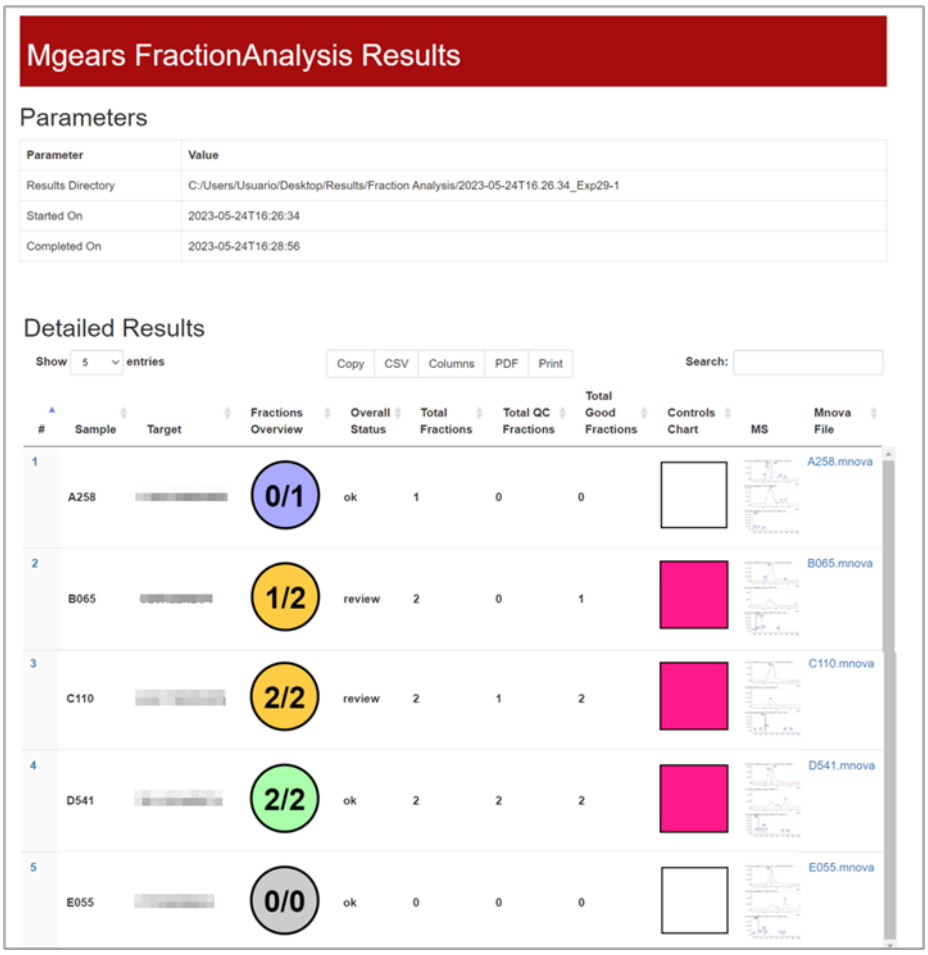
Once the configuration has been completed to your satisfaction, click on **Run** Run to launch the analysis.

3. The output folder

The results folder is saved under the previously specified directory, as described above.

3.1. The HTML file

The HTML report includes an overview of the results. Each sample is displayed on a row with the analyzed **Target** information (m/z, molecular structure), the **Overall status**, fraction results **(Total fractions**, **Total QC fractions**, **Total good fractions)**, a screenshot of the spectra, and links to the corresponding **Mnova** result files.



Mgears FractionAnalysis Results

Parameters

Parameter	Value
Results Directory	C:/Users/Usuario/Desktop/Results/Fraction Analysis/2023-05-24T16.26.34_Exp29-1
Started On	2023-05-24T16:26:34
Completed On	2023-05-24T16:28:56

Detailed Results

Show 5 entries — Copy | CSV | Columns | PDF | Print — Search:

#	Sample	Target	Fractions Overview	Overall Status	Total Fractions	Total QC Fractions	Total Good Fractions	Controls Chart	MS	Mnova File
1	A258		0/1	ok	1	0	0			A258.mnova
2	B065		1/2	review	2	0	1			B065.mnova
3	C110		2/2	review	2	1	2			C110.mnova
4	D541		2/2	ok	2	2	2			D541.mnova
5	E055		0/0	ok	0	0	0			E055.mnova

3.2. The CSV report

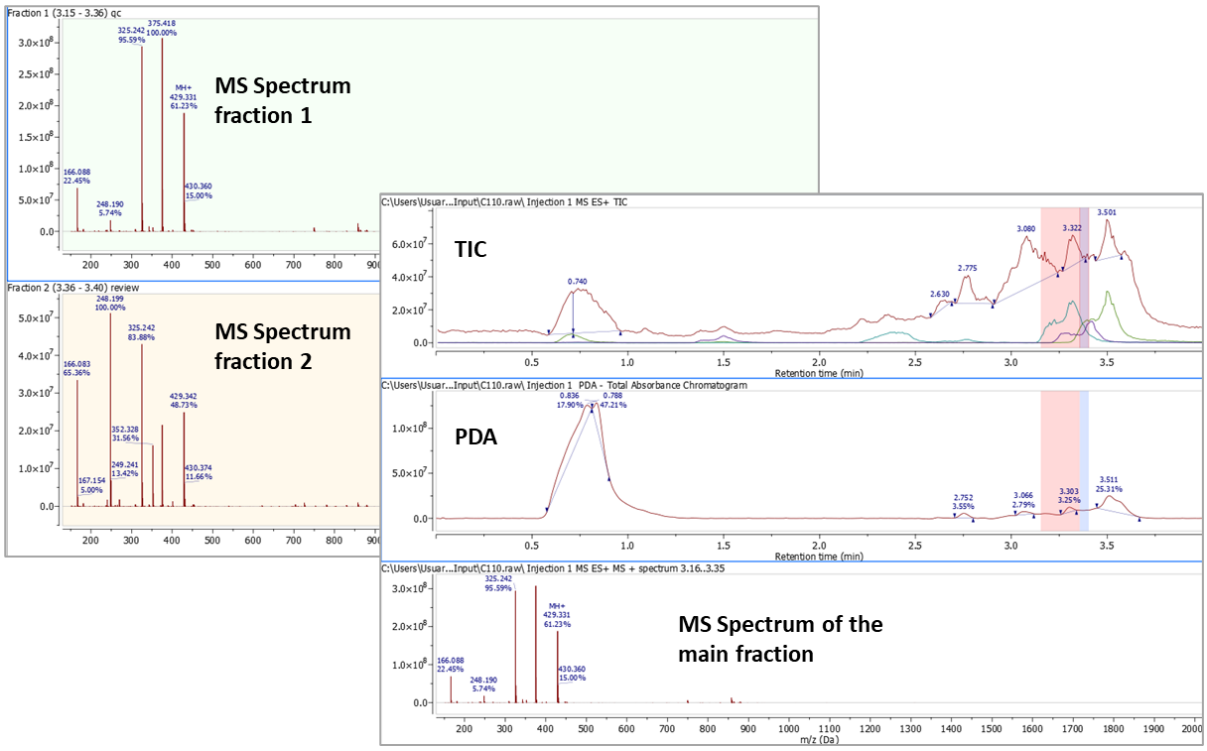
The CSV file saved under the “FractionAnalysis” subfolder contains the detailed results, such as the m/z ratio, number of chromatographic peaks, peak heights, MS purity, and the proposed Action for each analyzed fraction.

	A	B	C	D	E	F	G	H	I	J	K	L	M	N	O	P	Q
1	Sample	Target	MonoisotopicMass	TargetRT	Fraction	Vial	StartRT	EndRT	IonMZ	IonAdduct	Peaks	Height	HeightStatus	MSPurity	MSPurityStatus	Type	Action
2	A258		374.0606277	3.35	1	1,1:21	3.07	3.183	391.37	?	0	506733	low	7	low	Tail	dispose
3	B065		354.1579572	-	1	1,1:19	3.282	3.484	219.15	?	1	4.6E+07	high	0	low	Impure	review
4	B065		354.1579572	-	2	1,1:20	3.484	3.532	332.36	?	0	225226	low	0	low	Tail	dispose
5	C110		428.1769781	3.319	1	1,1:17	3.154	3.357	375.42	?	1	1.2E+07	high	19	low	Impure	review
6	C110		428.1769781	3.319	2	1,1:18	3.357	3.404	248.2	?	0	9221773	high	13	low	Tail	review
7	D541		351.1834437	3.314	1	1,1:14	3.137	3.34	248.2	fragment	1	5E+07	high	68	medium	Impure	qc
8	D541		351.1834437	3.314	2	1,1:15	3.34	3.387	248.2	fragment	2	6.1E+07	high	53	medium	Impure	review
9	X525		344.0864485	3.648	1	1,1:1	3.512	3.796	240.16	?	2	4.9E+07	high	0	low	Impure	review
10	Y562		335.0575912	1.898	1	1,1:22	3.333	3.536	296.19	?	1	1.5E+08	high	0	low	Impure	review
11	Y562		335.0575912	1.898	2	1,1:23	3.536	3.583	296.19	?	1	2.1E+07	high	0	low	Impure	review
12	Z190		394.0752289	-	1	1,1:16	3.35	3.541	313.22	?	1	9.3E+07	high	11	low	Impure	review

3.3. The PDF and Mnova

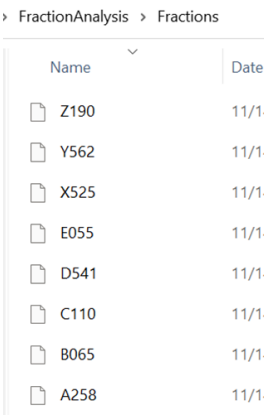
The PDF and Mnova reports have similar layouts and show:

- The Total Ion Chromatogram (TIC) with the fractions highlighted in colors (red and purple in the example below).
- The UV trace (PDA) as defined in the chromatogram extraction parameters.
- The MS spectrum of the main fraction with the MS peak assigned to target.
- The MS spectrum of each fraction, with a background color indicating the proposed action (green indicates that the fraction can be submitted for quality control, yellow indicates that the fraction needs review, and no color means the fraction must be disposed of).



3.4. The JSON files

For every analyzed fraction, a JSON file is automatically generated and stored in the “Fractions” subfolder within the "FractionAnalysis" folder. This file encompasses all the pertinent information obtained from the analysis, allowing customers to extract and utilize the data in any desired format for subsequent processes. The JSON file serves as a comprehensive resource, ensuring seamless integration and accessibility of relevant information for downstream operations.

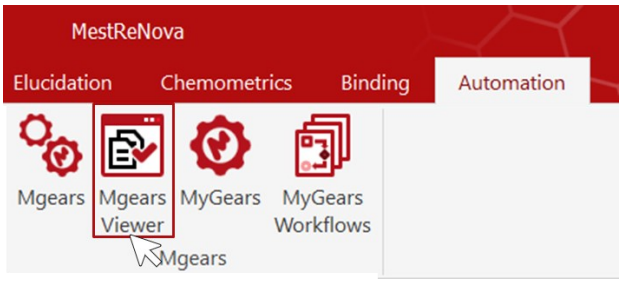


3.5. Other output

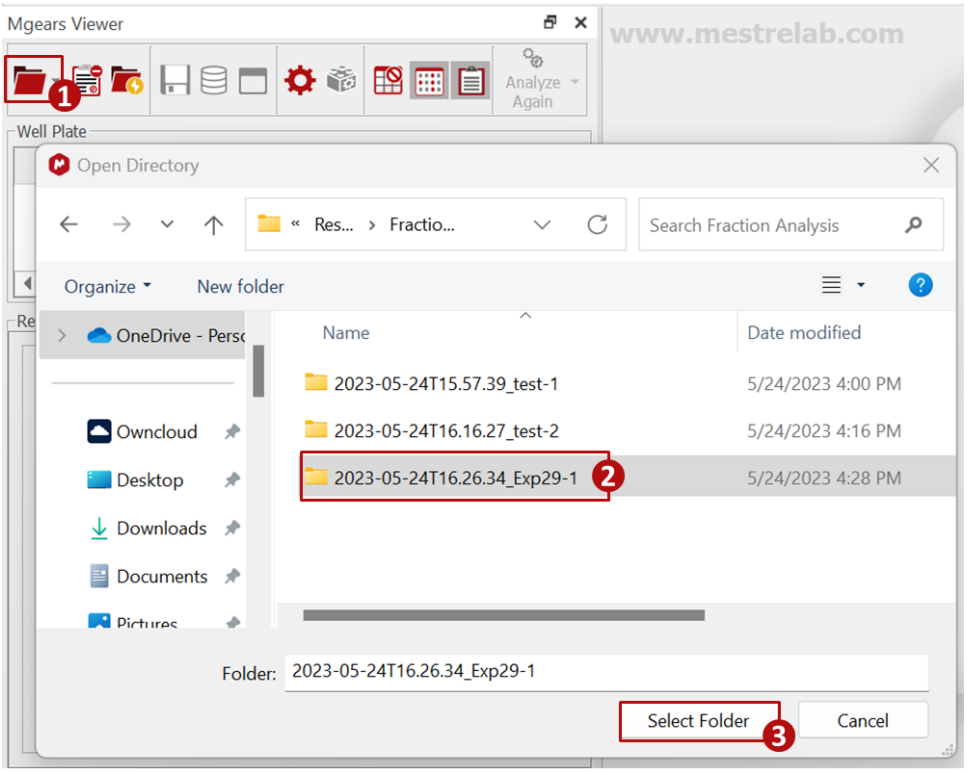
- A “documents” directory, containing the output Mnova files (unless Mgears is configured to save Mnova files to another location).
- A “pdf” directory, containing the output PDF files (unless Mgears is configured to save PDF files to another location).
- A log file of the execution.
- A copy of the settings used in the current evaluation.
- A resume file of the steps followed in the execution.
- A CSS folder, a data folder, a JS folder, and an images folder.

4. Mnova Gears Results Viewer

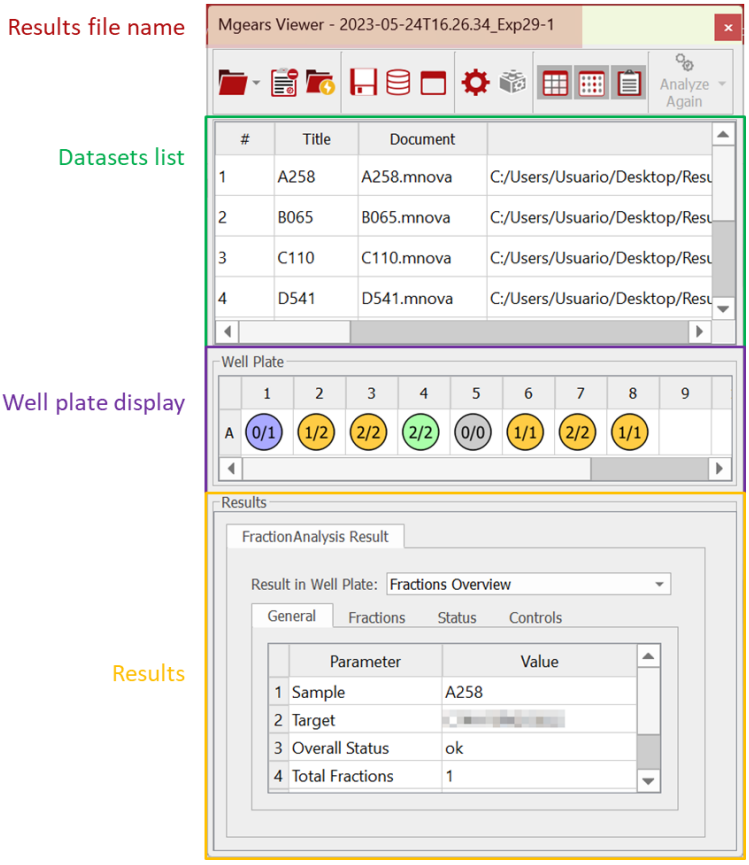
Fraction Analysis is compatible with the Mnova Gears Results Viewer. Open the **Mgears Viewer** from the Mnova **Automation** tab.



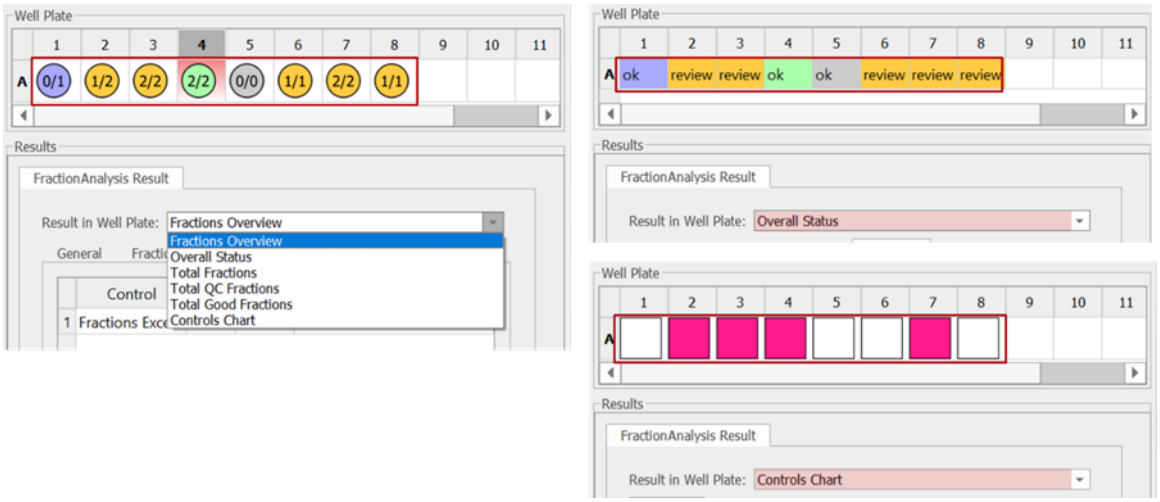
Click on  and select your analysis result folder to open it.



When the experiment is open, the **Mgears Viewer** displays a list of analyzed datasets, their positions in the well plate, and any associated numerical results.



The well plate can show graphically the results in different ways. It can either show the **Fractions Overview, Overall Status, Total Fractions, Total QC Fractions, Total Good Fractions,** or the **Controls Chart**.



The **Fractions Overview** states the number of fractions to keep (for QC or Review) / the total number of fractions collected.

Eg. 1/2 *means the sample has 2 fractions, 1 of which is to be kept for either QC or review.*

The well color can be either:

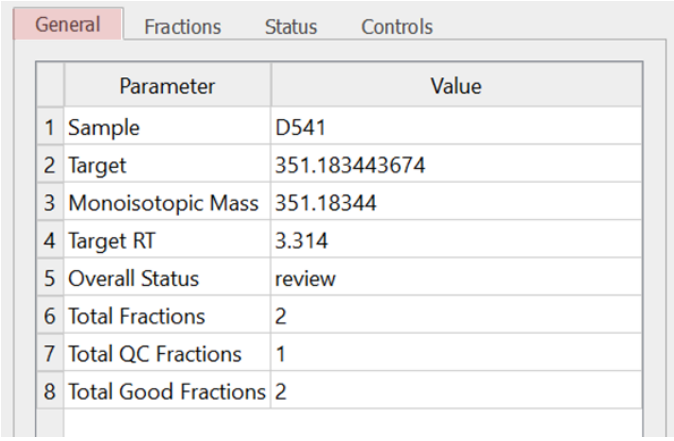
- **Grey**, which indicates that no fractions are collected.
- **Blue**, which indicates that no fractions should be kept.
- **Orange**, which indicates that at least one fraction requires review.
- **Green**, which indicates that all analyzed fractions are okay for quality control.

Click on a specific dataset to view the corresponding results and spectrum.

The **Results** section includes four tabs: General, Fractions, Status, and Controls tabs.

4.1. The General tab

This tab displays the overall results from the analysis.

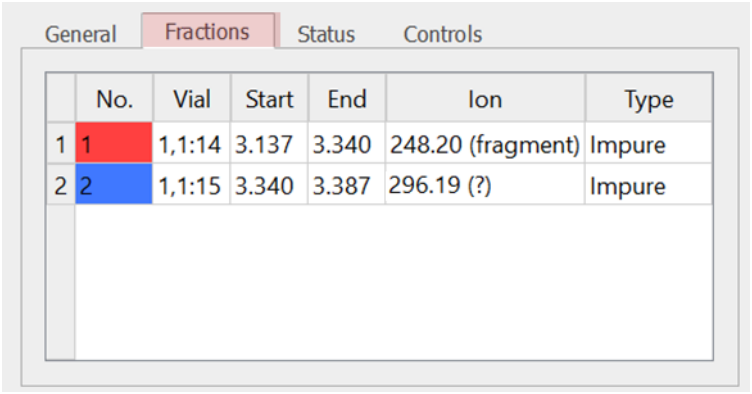


	Parameter	Value
1	Sample	D541
2	Target	351.183443674
3	Monoisotopic Mass	351.18344
4	Target RT	3.314
5	Overall Status	review
6	Total Fractions	2
7	Total QC Fractions	1
8	Total Good Fractions	2

4.2. The Fraction tab

In this tab, you can review detailed results for each collected fraction, including vial position in the rack where the sample was collected, start/end RTs for the collected fraction, the assigned main ion, and the type of fraction peak.

The **Ion** column displays the most intense m/z in the MS spectrum, and within parentheses, the type of ion is indicated based on the configuration in Molecule Match Settings: MH+, fragment, adduct, etc. If this ion is not identified under the analysis conditions, a "?" is displayed in parentheses.



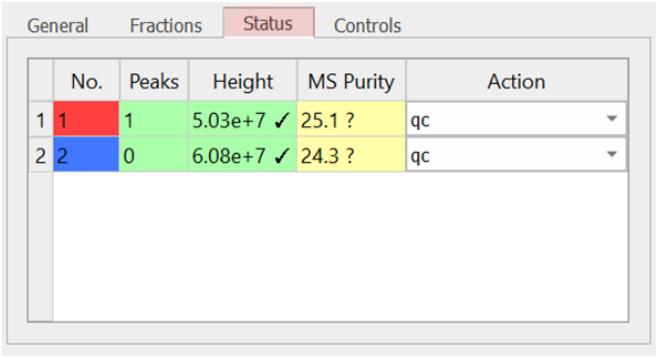
	No.	Vial	Start	End	Ion	Type
1	1	1,1:14	3.137	3.340	248.20 (fragment)	Impure
2	2	1,1:15	3.340	3.387	296.19 (?)	Impure

4.3. The Status tab

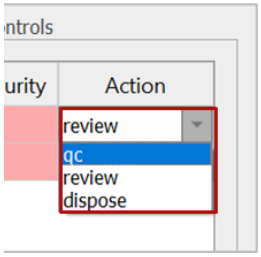
In this tab, you can inspect the scoring results for each collected fraction: the number of chromatographic peaks, their height, the MS purity, and the proposed action.

The score peak height and MS purity scores are determined based on the configured parameters as explained in the scoring scheme. The cells are color-coded and include symbols to represent the obtained score:

- "✓" and a **green** background indicate that the value is higher than the "high" threshold → The assigned score is HIGH.
- "?" and a **yellow** background indicate that the value is between the "low" and "high" thresholds → The assigned score is MEDIUM.
- "×" and a **red** background indicates that the value is lower than the "low" threshold → The assigned score is LOW.

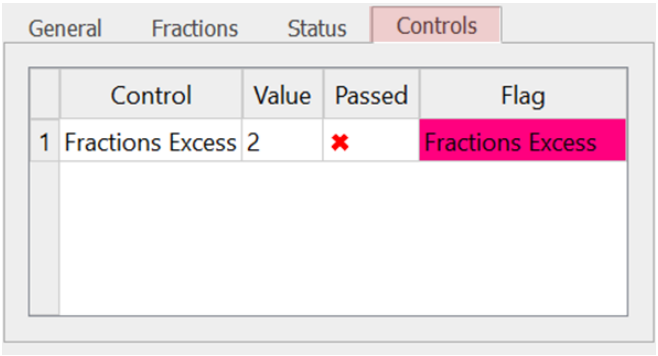


You have the option to manually override the assigned **Action** and choose another option. To do this, just click on the **Action** drop-down in the table and select your desired value. As you make this change, both the table and the well plate graphics will be updated accordingly.



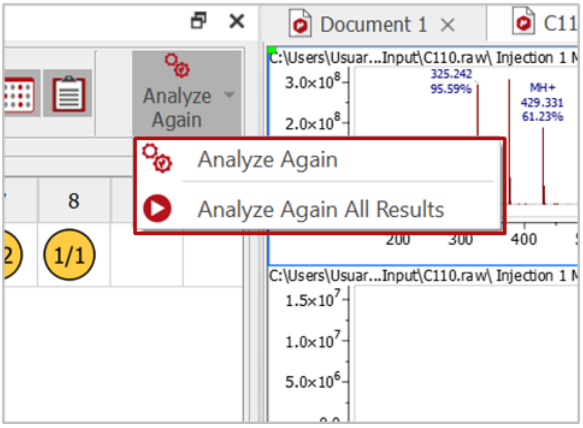
4.4. The Controls tab

In this final tab, the results of the controls are presented. The number of detected fractions is recorded in the **Value** column. When the number of collected fractions exceeds the predefined threshold, this is flagged with a color and message as customized in the plugin settings.



4.5. Results reviewing

Fraction Analysis results can be reviewed and edited when needed. You can correct peak picking, assignments, etc., and reanalyze your sample (**Analyze again**) or the whole dataset (**Analyze again all results**).



5. Fraction scoring and decision logic

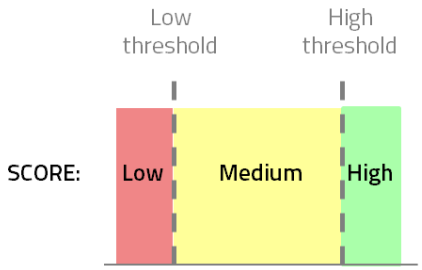
Fraction scoring relies on the evaluation of three criteria for each fraction:

1. **Mass Purity:** This is calculated as previously described and is categorized into one of three 'traffic light' regions (red, orange, green) based on the 'high/low' thresholds set in the brick.
2. **UV Response:** The UV (or other defined signal) for each fraction is assessed for height, either against an absolute or relative scale, depending on the brick's settings. Similar to mass purity, it falls into one of the three 'traffic light' regions according to the 'high/low' threshold settings.
3. **Number of Maxima in UV:** If the UV signal for a fraction contains more than one maximum, it suggests impurity, regardless of the mass purity. This could be due to higher time resolution in the UV or the presence of isomers.

These criteria collectively guide the brick's categorization of each fraction into one of the following categories:

- **'qc'**: This category designates fractions that are considered sufficiently pure to proceed into the next stages of the process, typically involving final quality control assessments.
- **'dispose'**: Fractions falling into this category contain minimal product or are highly impure. It is advisable to dispose of these fractions as combining them would likely reduce overall purity without significantly increasing yield.
- **'review'**: The statistical results for the fraction are unclear, and it is recommended that a human reviews the outcome.

The figure below illustrates how scores are evaluated and combined to define the action.



Purity	UV Peak Intensity	No. of peaks	Action
High	Medium/High	=1	QC
High	Medium/High	>1	Review
High	Low	=1 or >1	Review
Medium	Medium/High	=1 or >1	Review
Medium	Low	=1 or >1	Dispose
Low	High	=1 or >1	Review
Low	Low/Medium	=1 or >1	Dispose

For more detail on Mnova Gears' options, please refer to the Mnova Gears manual.